A STUDY OF THE VITAMIN NEEDS OF THE BABY PIG

BY

ALVIN LUDWIG NEUMANN

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AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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A STUDY OF THE VITAMIN NEEDS OF THE BABY PIG

The study of the vitamin requirements of the young pig has recently received considerable attention. Chick et al (1938) and Wintrobe (1939) fed purified diets to young pigs and were fairly successful when the pigs were initially from four to 12 weeks old. Johnson et al (1947) fed 2-day-old pigs on a purified "synthetic milk" diet and were able to produce very favorable growth rates, demonstrating the possibilities of this experimental technique.

The advantages of this experimental tool are obvious. Many of the uncontrolled factors present in raising the nursing pig are removed. Much greater quantities of the experimental diet are consumed than would be possible if the pigs nursed their dams, and the purified diet, with all its possibilities and advantages, may be used. Smaller total quantities of expensive purified diets are required when baby pigs are fed as contrasted to weanling or older pigs.

Some of the vitamin needs of the pig have been studied extensively, both qualitatively and quantitatively, but in most cases older pigs have been used and some experimental evidence indicates that the requirements of the young pig are higher and more critical than those of the older pigs.

In these investigations the choline requirement of the baby pig was studied both qualitatively and quantitatively. The need for unidentified growth factors by the baby pig also was investigated. Vitamin B_{12}, both in the crude concentrate and the crystalline form, was studied to determine if this vitamin is the unidentified growth factor. The relationship between vitamin B_{12} and peteroylglutamic acid was investigated because of the reported similarity of the response of human pernicious anemia patients to therapy of the two compounds.

The basal diets used in the six experiments conducted are shown in Table 1.

TABLE 1. COMPOSITION OF THE BASAL DIETS USED IN THE SIX EXPERIMENTS

INGREDIENTS	CASEIN DIET EXP. 1, 2, 3 (%)	ALPHA PROTEIN DIET EXP. 3, 4, 5, 6 (%)
Labco vitamin-free casein	30.0	
Alpha Protein		29.7
dl-methionine		0.3
Glucose (Cerelose)	30.94	30.94
Lard	30.76	30.76
Mineral salts	8.30	8.30

These materials plus water were formulated into a "milk" containing 13% solids and 4% fat. To each liter of this "milk" were added the following vitamins:

Thiamin	0.65 mg	A-tocopherol	
Riboflavin	1.30 "	acetate	1.00 mg
Pyridoxine	1.30 "	2-methyl-1,4-	
Calcium pantothenate	7.80 "	naphthaquinone	0.26 "
Inositol	26.00 "	Pteroyolutamic	
Nicotinic acid	2.60 "	acid[2]	0.052 "
P-amino-benzoic acid	2.60 "	Choline chloride[3]	130.00 "
Biotin	0.01 "	Vitamin A[4]	2000 I.U.
Ascorbic acid[1]	16.00 "	Vitamin D[4]	200 I.U.

[1] Not used in choline tests.

[2] Not included in basal diet of Vitamin B_{12}-pteroylglutamic acid test.

[3] This amount of choline added after requirement was established in choline experiment.

[4] Only one-half these amounts used in choline studies.

Choline Studies

Two experiments were conducted to study the role of and

need for choline. In the first test, using *ad libitum* feeding, 12 baby pigs were fed for eight weeks to establish the essentiality of the vitamin and the range of the requirement. In the second test, using paired feeding, six pairs of similar pigs were fed for the same period, to eliminate any effects of differences in diet consumption within the limits of this range. The study was made, recognizing the dual role of the vitamin and its relationship to methionine and cystine. The methionine content of the basal casein diet was calculated to be from 0.8 to 1.0 per cent. This is approximately the level of methionine found in complete weanling pig rations compounded from natural feedstuffs.

In the *ad libitum* test 0.10 per cent choline per unit of dry matter in the diet produced maximum gains whereas either no choline or 0.05 per cent choline produced poor growth and a lack of uniformity. Several of the choline deficient pigs showed characteristic gross symptons such as poor muscle tone, short-leggedness, pot-bellied conformation, and incoordinated movements.

The 0.05 and 0.10 per cent levels of choline were studied in the paired feeding test. No significant differences in growth rate or economy of gain were noted when equal amounts of diet were consumed by the pair mates. However, significantly more fat was deposited in the livers of the pigs on the 0.05 per cent level of choline and pathological conditions were found in the kidneys which were identical to those found in the deficient pigs of the *ad libitum* test, namely, glomerular occlusion and tubular epithelial damage. In both tests all pigs on the 0.10 per cent level of choline were normal in all respects.

Studies of Growth and Hematopoietic Factors, Including Vitamin B_{12}

Four tests were conducted to study growth and hematopoietic factors in baby pig nutrition. Cary and co-workers (1946) showed that young rats required a growth factor contained in commercial liver preparations and Ruegamer

et al (1947) found the same to be true with dogs.

In the first test the Alpha Protein basal diet plus Reticulogen (anti-pernicious anemia liver extract) was compared with the same diet, unsupplemented, and with the previously used casein diet. Significantly greater and more economical gains were made by the baby pigs receiving the liver extract supplement (0.33 ml. per pig daily) after eight weeks on test. The pigs fed the unsupplemented Alpha Protein basal diet grew slowly but were normal in gross appearance. Therefore, this basal diet plus methionine was used as a control diet in further studies of the unidentified growth factor(s) requirement of the baby pig.

To determine if a crude vitamin B_{12} concentrate would supplement the diet deficient in unidentified growth factor(s), 12 baby pigs were fed for 11 weeks on the Alpha Protein diet plus various levels of the available vitamin B_{12} concentrate which was prepared from liver. The diet of one group of pigs in this test was supplemented with an "animal protein factor" supplement prepared from a hen feces mold preparation. Both supplements exterted growth factor activity. A level of 51 mcg. of vitamin B_{12} potency per kilogram of dry matter in the diet was required to produce optimum gains. Complete and normal red blood cell maturation did not occur in any of the groups as indicated by a slight normocytic anemia and a hyperplastic marrow with an abnormally high percentage of the immature forms of the erythroid series cells, as well as of remnants of incompletely formed cells such as the Howell-Joli bodies.

Injected crystalline vitamin B_{12} was tested to determine its essentially by the baby pig and to compare its potency with Reticulogen. Twelve pigs were fed the Alpha Protein basal diet for a 21-day depletion period and then divided into a control group, a vitamin B_{12} group, and a Reticulogen group. Daily injection with either 2 mcg. of crystalline vitamin B_{12} or 0.10 ml. of Reticulogen significantly improved the growth rate and economy of gain over the unsupplemented group. The

magnitude of the response to the two supplements was identical and both induced normal hematopoiesis. Three pigs died on the unsupplemented basal diet after showing signs of appetite failure, anemia, diarrhea, and listlessness. Histological studies of various organs failed to characterize definitely the vitamin B_{12} deficiency.

West (1948) reported favorable responses by human pernicious anemia patients to vitamin B_{12} therapy, suggesting a similarity, or at least a relationship between vitamin B_{12} and pteroylglutamic acid.

A dual deficiency of vitamin B_{12} and pteroylgultamic acid was produced in 12 baby pigs by first feeding the vitamin B_{12}-deficient Alpha Protein basal diet for 21 days and then feeding crude methyl folic acid, a vitamin antagonist, for an additional 14 days. Five pigs died during this period or shortly afterward with combined deficiency symptoms. Vitamin B_{12} effected a complete cure and restored appetite with consequent optimum gains within 21 days after theraupeutic treatment began. Pteroylglutamic acid therapy induced normal hematopoiesis but failed to stimulate and maintain normal growth.

Conclusions

Within the limits of the conditions imposed in this study, the following conclusions seemed justified:

Choline Studies

1. On a "synthetic milk" diet containing approximately 0.80 to 1.00 per cent methionine (30 per cent casein), the baby pig requires dietary choline.

2. The level of essential dietary choline required by the baby pig on a diet containing approximately 0.9 per cent methionine, which is a level comparable to that found in well-balanced natural diets, is more than 0.05 per cent but not more than 0.10 per cent of the dry matter in the diet. The requirement is probably nearer the larger figure.

3. It appears that, in the young pig, as in the chick, the ability of methionine to supply methyl groups for the synthesis of choline from aminoethanol may be limited. The level of methionine in the experimental diet would have to be raised to a level which would be impractical in actual practice.

4. Choline deficiency in the baby pig results in gross symptoms of unthriftiness, poor conformation (short-legged and pot-bellied), lack of coordination in movements and a characteristic lack of proper rigidity in the joints, particularly in the shoulders, and a general lack of muscle tone. The histological symptoms are a typical fatty infiltration of the liver and a characteristic renal glomerular occlusion, with some tubular epithelial necrosis, but no hemorrhagic condition.

5. When the feed intake is equalized, choline at the 0.05 and 0.10 per cent levels does not have a growth-stimulating effect. However, if *ad libitum* feeding is practiced, the pigs on the 0.10 per cent level of choline voluntarily consume more diet and consequently gain more rapidly.

Studies of Growth and Hematopoietic Factors, Including Vitamin B_{12}

1. A "synthetic milk" whose protein source was Alpha Protein, an isolated soybean protein, was found to be a suitable control diet in studying the need of the baby pig for unidentified growth factor(s).

2. Compared with a casein diet, the Alpha Protein diet apparently contains less of the unidentified growth factor(s).

3. Reticulogen, fed at the rate of 0.25 or 0.33 ml. per pig daily, supplies enough of the unidentified growth factor(s) to support normal growth and maintain a normal blood picture.

4. The similarity in magnitude of the responses produced by Reticulogen when added to either the casein or the Alpha Protein diet suggests that the diets had identical or related deficiencies.

5. Favorable growth responses were obtained when sufficiently high levels of a vitamin B_{12} crude concentrate[1] were added to the basal Alpha Protein diet which is known to be devoid or low in unidentified growth factor(s).

6. When enough vitamin B_{12} crude concentrate was added so that the diet contained active material equal in potency to 51 mcg. of crystalline vitamin B_{12} per kilogram of dry matter in the diet, optimum growth rates were obtained. The addition of more crude concentrate did not result in a further response.

7. Even the highest levels of vitamin B_{12} crude concentrate fed did not induce normal hematopoiesis, as indicated by the presence of a normcytic anemia, by an abnormally high erythrocyte count in the marrow, and by the presence of large numbers of the various forms of immature cells in the marrow.

8. Gross symptoms which might be attributed to the deficiency existing in the basal diet were hyper-irritability, dullness, voice failure, posterior incoordination, and a pain in the rear quarters.

9. The crude concentrate containing an active material synthesized by microorganisms isolated from hen feces[2] partially met the growth requirements for unidentified growth factor(s) when added to the basal diet at the level fed.

10. Vitamin B_{12} is required by the baby pig.

11. Crystalline vitamin B_{12} and Reticulogen, when injected at levels to supply equal amounts of vitamin B_{12} potency, produce equal responses in growth rate and both produce normal pigs. The equality in growth response indicates that

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[1] Courtesy of Dr. Tom Jukes, Lederle Laboratories, Pearl River. N. Y.

[2] Courtesy of Dr. D. F. Green, Merck, Inc., New York. This material was reported to contain 2 mg. of vitamin B_{12} potency per pound.

vitamin B_{12} is the growth factor being supplied by the anti-pernicious anemia liver extract preparation.

12. Crystalline vitamin B_{12} was the growth factor lacking in the basal Alpha Protein diet.

13. Intramuscularly injected crystalline vitamin B_{12} alleviated a deficiency of both pteroylglutamic acid and vitamin B_{12} when used at the rate of 2 mcg. per pig daily by producing optimal growth and normal hematopoiesis in baby pigs.

14. Pteroylglutamic acid alone temporarily resulted in a sub-optimal growth response with an optimal, sustained response in hematopoiesis. The simultaneous administration of both vitamin B_{12} and pteroylglutamic acid produced and sustained both optimal growth and hematopoiesis.

15. The crude methyl folic acid used as the pteroylglutamic acid antagonist in this test apparently produced deleterious effects in addition to acting as the vitamin antagonist.

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VITA

Alvin Ludwig Neumann was born July 9, 1915, and reared on a farm near Granite, Oklahoma. He attended Ozark Consolidated School near Granite, Oklahoma, for 12 years, graduating in 1933. He farmed with his father until July, 1934.

He entered Oklahoma A. and M. College in September, 1934, and graduated with the degree of Bachelor of Science in June, 1938. He served as an assistant in the Agriculture Education Department in the same school and received the degree of Master of Science in June, 1939, with a major in Animal Husbandry.

From July, 1939, to June, 1941, he taught vocational agriculture at Cooperton, Oklahoma, High School. From July, 1941, to August, 1944, he was head of the Agriculture Department and Professor of Animal Husbandry at Cameron State Agriculture College at Lawton, Oklahoma. He served as County Farm Agent of Comanche County, Oklahoma, from August, 1944, to August, 1945, when he became an Assistant Professor of Animal Industry at the University of Arkansas, Fayetteville, Arkansas.

He entered the University of Illinois Graduate School in June, 1947, to work towards the degree of Doctor of Philosophy, and served as an assistant in the Animal Science Department from September, 1947, to September, 1948. His graduate work consisted of a major in Animal Science, first major in Animal Nutrition, and second minor in Physiology.

He was married to Miss Lorena Petermann, Stillwater, Oklahoma, on June 12, 1938.